

In the Specification:

Please amend the specification as shown:

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8/14/08
Please delete the paragraph on page ²⁰18, line 28, through page ²¹20, line 7, and replace it with the following paragraph:

Figure 1: (SEQ ID NOS 32-34 respectively in order of appearance) Direct binding of GEF-H1 to PAK4. Lysates were prepared from 293T cells over expressing (a) myc tagged PAK4 alleles or subdomains and incubated with GST, GST-GEF-H1 (787-921) and GST-Maguin-like bound to Glutathione beads (1,2,3,4 bead designation) or (b) GFP-GEF-H1 and incubated with PAK4 biotinylated peptides (291-355) and (276-324) linked to streptavidin beads. An equivalent amount of beads and excess lysates (500ug) were used. Eluates of beads and total lysates were analysed by SDS-PAGE and immunoblotted with myc or GEF-H1 antiserum; (c) represents a PAK4 gene schematic outlining the regions of PAK4 used to identify the GEF-H1 interaction domain (GID). d, alignment of the GID defined by the minimal binding region of PAK4 with PAK5 and PAK6 .

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Please delete the paragraph on page ²¹20, line 19, through page ²²21, line 2, and replace it with the following paragraph:

Figure 3: (SEQ ID NOS 35-37, 37-45 and 45-47, respectively in order of appearance) PAK4 phosphorylates GEF-H1 *in vitro* and *in vivo*. (a) *In vitro* kinase assay shows that purified GST, GST-GEF-H1(763-921) and GST-Maguin-like protein are phosphorylated by PAK4. The negative control autophosphorylation reaction is the reaction mix without substrate. A concentration gradient of reaction substrate is indicated by the triangles; (b) A weighted PAK4 substrate consensus aligned to phage display hits(above the broken line). Asterisk indicates the phospho-acceptor site. An amino-terminal GEF-H1 peptide (below broken line) with a predicted phosphorylation site is marked with "***"; (c) *In vivo* phosphorylation of GEF-H1 by PAK4 is demonstrated in a western blot of co-transfected lysates were probed with

phosphospecific anti-HA antisera (lower panel). The amount of total GEF-H1 was detected with HA antiserum (upper panel).

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Please delete the paragraph on page ²³22, lines 15-20, and replace it with the following paragraph:

Figure 8. (SEQ ID NOS 18, 3, 3, 3, 4, 4 and 4, respectively in order of appearance) Phosphorylation scores of GEF-H1 proteins and peptides. (a) Peptide phospho-isomers derived from potential regulatory regions, used to confirm phosphorylation sites. Residues printed in red are prephosphorylated and block phosphorylation *in vitro*; (b) A combination of deletion and site-directed mutants used to physically located PAK4 phosphorylation on the GEF-H1 protein.

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Please delete the paragraph on page ²³22, lines 22-26, and replace it with the following paragraph:

Figure 9. (SEQ ID NOS 35, 48-49, 35 and 50-51, respectively in order of appearance) Conservation of GEF-H1 with Cdc24. Conservation of residues with Cdc24 are shaded in pink and yellow. PAK4 phosphorylation sites are highlighted by asterisks on the weighted consensus is which is aligned to the putative regulatory regions conserved with Cdc24.

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Please delete the paragraph on page ²⁷26, lines 1-14, and replace it with the following paragraph:

This information leads to the prediction that a phosphorylation consensus sequence for GEF-H1 is, from N-terminus to C-terminus, "RBSZXG" (SEQ ID NO. 6) or "RBSZXL," (SEQ ID NO. 20), where R is arginine, B is a basic amino acid, S is serine, Z is a hydrophobic amino acid, X is any amino acid, G is glycine, and L is leucine. At some phosphorylation sites in GEF-H1, the C-terminal amino acid residue is a glycine,

while at other sites, the residue is a leucine. Accordingly, any peptide comprising this consensus sequence structure is potentially a target for phosphorylation by PAK4, and such peptides can be used in assays according to the present invention. Furthermore, any peptide that consists essentially of the RBSZXG (SEQ ID NO: 6) and/or the RBSZXL (SEQ ID NO: 20) consensus sequences is potentially a target for phosphorylation by PAK4, and such peptides can be used in assays according to the present invention.

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8/14/08 Please delete the paragraph on page ³¹~~30~~, line 20, through page ³²~~31~~, line 2, and replace it with the following paragraph:

The present invention uses a biochemical-based cell lysate assay and a radioactive method to monitor, record and detect changes in phosphorylation of total GEF-H1 protein and GEF-H1-derived peptides by PAK4. See Examples 14 and 15 below. In the cellular assay, phosphospecific antibodies to GEF-H1 are used to detect the presence of phosphorylated GEF-H1 in cell lysate preparations. Antibodies that are specific to GEF-H1 can be generated by any one of a number of techniques. For example, antibodies can be produced in rabbits. See, Nims *et al.*, *Lab Anim. Sci.*, 23(3):391-6, 1973. Antibodies can be raised against epitopes that are unique or specific to a particular protein. For instance, one may raise antibodies against the KLH-conjugated peptide CSGDRRRAGPEKRPKSS (SEQ ID NO: 23), as previously demonstrated for PAK4. See, Hashimura *et al.*, *J. Immunol. Methods*, 55(3):375-87, 1982.

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8/14/08 Please delete the paragraph on page ⁴⁴~~43~~, line 26, through page ⁴⁵~~44~~, line 11, and replace it with the following paragraph:

Previously, it was found that peptides derived from the activation loop sequence of PAK4 are high affinity substrates of PAK4. By examining the ability of PAK4 to phosphorylate a series of peptides highly related to its activation loop, it was possible to deduce a rough substrate consensus for PAK4 (RRXSL(X)nG (SEQ ID NO: 24),

where $n = 1$ or 2) that suggested that both the basic residues and the hydrophobic residues flanking the phospho-acceptor site are important for high affinity substrate recognition by PAK4. To further explore the substrate selectivity of PAK4, the sequences of the phage display clones enriched in the PAK4 kinase domain screen were compared with our putative PAK4 substrate consensus. Most of the sequences, including GEF-H1S, contain a region with high similarity to a high affinity PAK4 substrate. Within the GEF-H1 phage display hit that corresponds to GEF-H1S amino acids 763-921, a potential PAK4 phosphorylation site was identified at Ser 810 of GEF-H1S. A peptide derived from the sequence surrounding this site (amino acids 807-824) is also a high affinity substrate for PAK4 with a similar K_m for PAK4 as the GST-GEF-H1 (793-921) fusion protein.

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Please delete the paragraph on page 44, lines 13-15, and replace it with the following paragraph:

Thus, a substrate consensus motif for PAK4 mediated phosphorylation is identified herein as $RRXSL(X)_nG$ (SEQ ID NO: 24), where n is "1" or "2" X residues, and "X" is any amino acid.

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Please delete the paragraph on page 58, lines 5-12, and replace it with the following paragraph:

From the phosphorylation experiments described above, two peptides of GEF-H1S were identified as being targets for PAK4-induced phosphorylation. The GEF-H1S target residues were identified by aligning the PAK4 activation loop peptide with the GEF-H1S polypeptide sequence amino acids 762 to 921. *In vitro* kinase assays subsequently showed that GEF-H1S peptides PRRRSLPAGDALYLSFNPP (SEQ ID NO. 3 45) and RQSLGSRGRSSLSLAK (SEQ ID NO. 4) are phosphorylated by PAK4.

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8/14/08 Please delete the paragraph on page ⁵⁹~~58~~, lines 14-22, and replace it with the following paragraph:

The extent of phosphorylation of these peptide substrates was compared visually by autoradiography and a consensus of substrate phosphorylation empirically determined. A consensus sequence could predicted to be, for instance, **RBSZX[G/L] (SEQ ID NO: 25)**, where R is the amino acid arginine; B is any basic amino acid; S is the amino acid serine; Z is any hydrophobic amino acid; X is any amino acid, G is glycine and L is leucine. Identification of the amino acid residue was performed by kinase assay using PAK4 as the enzyme and isomers of predicted substrate regions of GEF-H1S.

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8/14/08 Please delete the paragraph on page ⁶¹~~60~~, lines 11-17, and replace it with the following paragraph:

Antibodies specific for PAK4 were generated in rabbits by immunizing rabbits with the KLH conjugated peptide CSGDRRRAGPEKRPKSS (**SEQ ID NO: 23**), which is part of the PAK4 protein, conjugated to KLH. See, Nims *et al.*, 1973. 23(3):391-6; and Hashimura *et al.*, 1982. Antibodies specific for GEF-H1 were also generated in rabbits by immunizing with the GST-GEF-H1S polypeptide which comprises amino acids 762 to 921.

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8/14/08 Please delete the paragraph on page ⁶⁷~~66~~, lines 17-27, and replace it with the following paragraph:

To obtain GEF-H1S, the oligonucleotides MC1 GCAGAATTCTGTAACAAGAGCATCACA (**SEQ ID NO: 16**) and MC3b GCGCTCGAGTTAGCTCTCGGAGGCTACAGCCT (**SEQ ID NO: 17**), derived from sequence KIAA0651, were used in 20 rounds of Polymerase Chain Reaction (PCR) to amplify the gene from a human whole brain plasmid cDNA library (CLONTECH laboratories Cat# HL9002CC). A ~3000 bp fragment was obtained, subcloned and

three clones sequenced yielding identical results. An EcoR1- Xho1 was used for subcloning in frame into a pcDNA vector harbouring the HA-epitope 5' to the MCS. EcoR1 - Xho 1 fragment from pcDNA clones were used for shuttling all GEF-H1 alleles to in frame Eco-R1-Sal 1 digested pEGFPC (Clontech) respectively.

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8/11/08 Please delete the paragraph on page ⁶⁷~~66~~, line 29, through page ⁶⁸~~67~~, line 19, and replace it with the following paragraph:

Mutant S810A was introduced by PCR reaction with oligos MC27
TG TAGATCCTCGGCGGCGCGCTCTCCCGCA (SEQ ID NO: 10) and MC3b. The mutant insert was introduced to a deletion mutant of GEF-H1S spanning nucleotides 1100 - 2761 and wild type BamH1 -Xho1 fragment was substituted with mutant XhoII and XhoI fragment. Oligonucleotides MC40
GGTGATGCCCATGGTCTCCAGCAGGAT (SEQ ID NO: 11), MC1, MC 41
ATCCTGCTGGTGACCATGGGCATCACCA (SEQ ID NO: 12) and MC3b were used to create overlapping EcoR1-Nco1 and Nco1-Xho1 fragments to convert QR residues to MG in the highly conserved QRITY motif of the DH domain. S67A was created using oligos MC57 GCCGTGGCCGCTCCGCCTTGTCTTTA (SEQ ID NO: 13) and MC58 TAAAGACAAGGCGGAGCGGCCACGGC (SEQ ID NO: 14) and pBluescript GEF-H1S spanning 1- 626 as a backbone in a site directed mutagenesis reaction (Stratagene). This mutant fragment was digested with EcoR1 - Sac1 and joined to the remainder of the gene with a Sac-Xho1 generated fragment from mutant and wild type alleles. Since GEF-H1M and S proteins differ only in their amino termini it was generated as a hybrid by joining mutant and wild type sequences derived from GEF-H1S. The unique 5' sequence was generated with the oligonucleotides MC61
GCGGAATTCATGTCTCGGATCGAATCCCTCA (SEQ ID NO: 26) and MC62
GTCAGTGAAGCTCGTCCACGCAGGGGA (SEQ ID NO: 27) in a PCR reaction using IMAGE clone 4157775(Research genetics) as a template.

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8/14/08 Please delete the paragraph on page ⁶⁸67, line 24, through page ⁶⁹68, line 3, and replace it with the following paragraph:

Glutathione-s-transferase (GST) fused PAK4 (residues 291-591), GEF-H1 amino acids 763-921 (from phage clone 13.8 sequence, matches KIAA0651) and Maguin 2-like (from phage clone 13.32, sequence matches accession # XP-087831) were purified by standard procedures and as previously described 31. Amino acid sequence of peptides spanning GEF-H1b 807-824 #1008 (RRRSLPAGDALYLpSFNPP) (SEQ ID NO: 28), #1009 (RRRpSLPAGDALYLSFNPP) (SEQ ID NO: 29), #1010 (RRRSLPAGDALpYLSFNPP) (SEQ ID NO: 30) and #934 (RRRSLPAGDALYLSFNPP) (SEQ ID NO: 3) used for in vitro kinase assays. In vitro kinase reactions were run as described previously 31.

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8/14/08 Please delete the paragraph on page ⁶⁹68, lines 7-22, and replace it with the following paragraph:

KLH linked peptide (#1009) CRRRSLPAGDALYLSFNPP (SEQ ID NO: 52) (residues 807-824) with a phosphorylated serine in position 810 and (#104) GST-GEF-H1 (residues 762-921) were used as antigens to raise anti-serum in rabbits. PAK4 antibodies were derived from the antigens (#933) CATTARGGPGKAGSRGRFAGHSEA (SEQ ID NO: 31) (residues 122 – 144) and (#80) CSGDRRRAGPEKRPKSS (SEQ ID NO: 23) (residues 148 – 163). Specificity was analysed as described previously. Goat anti-mouse and anti-rabbit IgG horseradish peroxidase conjugates were from Roche molecular. Goat anti-mouse and anti-rabbit linked to fluorophores FITC or rhodamine were from Santa Cruz biotechnology and Mouse anti- β -tubulin was from Zymed. Coumarin-phalloidin and Phalloidin-FITC were from molecular probes. Mouse anti-cascade blue and the fluorescent dyes; Texas red, FITC or marina blue were obtained and conjugated to antibodies according to the manufacturer. The solutions of cross-linking reagent were quenched by desalting through G25 sephadex and mixing with ammonium chloride to a final concentration of 50mM.